Effect of Hydrodynamic Pressure Processing on the Processing and Quality Characteristics of Moisture-Enhanced Pork Loins

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ABSTRACT: The objective of this study was to determine the influence of hydrodynamic pressure processing (HDP) and aging on the processing characteristics and final meat quality of moisture-enhanced pork loins. Boneless pork loins (n = 24) were split into 3 portions and assigned treatments: control (non-HDP treated, brineinjected), HDP treated before brine-injection, or HDP treated after brine-injection. Pork loins were injected with a salt/phosphate/water solution to 110% of original weight on day 0, intermittently tumbled 3 h, and then held overnight. Meat quality and protein characteristics were measured on days 1 and 8. HDP-treated loins had greater (P < 0.05) brine retention after overnight equilibration and a higher (P < 0.05) processing yield than controls. Warner-Bratzler shear force and expressible moisture decreased (P < 0.0001) with aging from days 1 to 8, but were not significantly affected by either HDP treatment. When the drip loss data from HDP treatments were pooled, HDP samples had lower drip loss values than controls. L* and b* measurements exhibited significant HDP by aging interaction effects, but a* was not influenced by either HDP or aging. Myofibrillar protein solubility and gel electrophoresis measurements of protein degradation were influenced by aging treatments. Data from this study suggest that HDP may have beneficial effects on the processing and final product quality of moisture-enhanced pork loins.

Practical Application: This study demonstrates that hydrodynamic pressure processing (HDP) is an effective postharvest technology for improving the processing and meat quality characteristics of moisture-enhanced pork loin products, benefiting both meat processors and consumers.

Keywords: aging, hydrodynamic pressure processing, meat quality, moisture enhanced, pork loin

Introduction

Product uniformity, consumer appeal, and ultimately the value of muscle foods can be impacted. of muscle foods can be improved through the utilization of various meat processing techniques. Brine-injection and marination are commonly used to produce moisture-enhanced whole muscle products. It is well established that the juiciness, tenderness, shelflife, and consistency of pork improves with the incorporation of a salt-phosphate brine (Papadopoulos and others 1991; Cannon and others 1993; Sutton and others 1997; Sheard and others 1998; Prestat and others 2002a, 2002b; Robbins and others 2002; Davis and others 2004). For processors, the use of brine-injection and marination technologies in whole muscle cuts improves final product yield.

Relatively little is known about the effects that brine-injection and marination have on meat that has been treated with high pressure. Hydrodynamic pressure processing (HDP) is a postharvest, high-pressure technique that has been shown to improve tenderness in nonenhanced pork loins (Moeller and others 1999; Bowker and others 2010). HDP utilizes high-pressure shock waves passing through water to tenderize vacuum-packaged meat. Besides causing instantaneous tenderization, HDP has been shown to enhance

aging tenderization in whole muscle products (Solomon and others 2002; Bowker and others 2008a, 2010). Treatment with HDP was shown to have minimal influence on meat quality attributes such as color and water-holding capacity (WHC) in nonenhanced pork loins (Bowker and others 2010).

While the impact of HDP on nonenhanced muscle cuts is well established, the effect of HDP treatment on brine-injected muscle products is currently unknown. In nonenhanced beef striploins, HDP has been shown to disrupt muscle ultrastructure by causing fragmentation in the I-band regions adjacent to the Z-lines of the sarcomere structure within myofibrils (Zuckerman and Solomon 1998). The tenderizing effect observed with HDP treatment of meat is the result of this physical alteration in the structural integrity of the myofibrils. Because muscle ultrastructure plays a key role in determining both water-holding and textural properties in meat, it is hypothesized that HDP-induced changes in muscle ultrastructure may improve the processing characteristics and final meat quality attributes in a moisture-enhanced muscle product. Thus, the objective of this study was to determine the effects that HDP treatment has on the processing characteristics and final meat quality of moisture-enhanced pork loins when applied to the muscle either before or after brine-injection and vacuum tumbling.

Materials and Methods

Muscle samples

At the Beltsville Agricultural Research Center abattoir, pork loins (longissimus dorsi) were removed from one side of 24 pork carcasses at 0.5 h postmortem. The boneless loins (IMPS 413E, USDA

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 $2 \,^{\circ}$ C for 6 h, and then frozen at $-15 \,^{\circ}$ C until use.

Experimental treatments

Loins were thawed at 2 °C for 48 h prior to the day of treatment (day 0). Each loin was divided into 3 sections that were randomly assigned to one of the following treatments: (1) brine-injected, vacuum tumbled controls; (2) HDP treatment before brine-injection and vacuum tumbling; or (3) HDP treatment after brine-injection and vacuum tumbling.

The loin sections assigned to HDP were individually packaged in boneguard bags (Cryovac© /Sealed Air Corp., Duncan, S.C., U.S.A.) and placed on a 40-cm dia × 1.3-cm thick metal reflector plate fitted on the bottom of a 98-L plastic container filled with water (4 to 6 °C). A 100 g cylindrical shaped binary explosive was detonated 31 cm above the meat to generate a high-pressure shock wave. Two loin sections were treated per HDP detonation.

The brine for all treatments was formulated to contain 3% sodium tripolyphosphate (Curafos STPP Sodium Tripolyphosphate Food Grade, Innophos, Cranberry, N.J., U.S.A.) and 2.0% salt in 2 °C water. All loin sections were injected to 110% of the green weight with a 3-needle injector attached to a 1/3 hp brine pump (model 6K298B, Koch Supplies, Kansas City, Mo., U.S.A.). Loin sections from all 3 treatments were vacuum tumbled together at 4 rpm (model ET-3, Sipromac, Quebec, Canada) using a 10 min on, 10 min off intermittent cycle for 3 h at 4 °C. After vacuum tumbling, loins were transferred to covered plastic containers and equilibrated overnight at 2 °C. Samples designated for HDP treatment after processing were HDP treated following the overnight equilibration.

On day 1, loin sections were cut into 2.5-cm thick chops that were randomly assigned to days 1 and 8 analyses of shear force, WHC, and color measurements. WHC and color measurements were made on the same chop. One 2.5-cm thick chop was cut for texture profile analysis on day 2. Additional 1.3-cm thick chops were cut for protein measurements (days 1 and 8) and drip loss determination. All samples were individually vacuum packaged and stored at 4 °C.

Processing parameters

Weights of individual loin sections were recorded preinjection (wt1), postinjection (wt2), immediately posttumbling (wt3), and following overnight equilibration (wt4). From these weights, the following processing parameters were determined:

Injection amount (%) = $[(wt2 - wt1)/wt1] \times 100$ Brine retained at the end of tumbling (%) $= [(wt3 - wt1)/wt1] \times 100$ Brine lost during equilibration (%) = $[(wt3 - wt4)/wt3] \times 100$ Processing yield (%) = $(wt4/wt1) \times 100$

Warner-Bratzler shear force and texture profile analysis

Prior to cooking, chops were chilled to 2 to 5 °C. Chops for peak shear force and texture analysis were cooked on an electric grill (model GGR50B, Salton Inc., Mt. Prospect, Ill., U.S.A.) to an endpoint temperature of 71 °C following guidelines of AMSA (1995). The internal temperature of chops was monitored using a Type J thermocouple attached to a temperature meter (model

1997) were trimmed of external fat, vacuum-packaged, chilled at HH21, Omega Engineering, Stanford, Conn., U.S.A.). The chops were turned once when the internal temperature was halfway between the initial and endpoint temperature. Cooking time and yield were recorded for each chop. Chops were cooled to room temperature for Warner-Bratzler shear force (WBSF) and texture profile analysis (TPA) tests. WBSF was determined by using a 1.3-cm dia coring tool to remove a minimum of 6 cores parallel to the direction of the muscle fibers that were sheared perpendicular to the muscle fibers using a Warner-Bratzler meat shear fixture on a texture analyzer (model TMS-90, Food Technology Corp., Sterling, Va., U.S.A.). For TPA, a 2.5-cm dia coring tool was used to remove at least 3 cores perpendicular to the chop surface and then compressed twice to 50% of the original height on a Universal Instron Testing Machine (model 1122, Instron Corp., Canton, Mass., U.S.A.) at a crosshead speed of 50 mm/min using a 7.5-cm dia compression platen. TPA parameters calculated were hardness, cohesiveness, springiness, and chewiness (Bourne 1978).

Expressible moisture, drip loss, and total moisture

Expressible moisture (EM) was measured on days 1 and 8 samples according to a modified procedure of Boles and Shand (2001). Four 1.3-cm dia cores were removed from each chop parallel to the muscle fibers and trimmed to 1.5 ± 0.2 g. The cores were placed on filter paper in a 50-mL tube and centrifuged at $2400 \times g$ for 20 min at 4 °C. EM was calculated as the percent of water removed from the sample [((initial wt – centrifuge wt)/initial wt) $\times 100$].

Gravitational drip loss was determined on duplicate 20 to 30 g meat samples removed from a 1.3-cm thick chop on day 1. Initial weights were recorded and samples were suspended in sealed plastic containers and stored at 4 °C. After 24, 96, and 168 h, samples were blotted and reweighed (final weight). Drip loss percentage was calculated as follows: [(initial wt – final wt)/initial wt] \times 100.

Total moisture in meat samples was measured in triplicate according to AOAC procedure 950.46B (1990).

Color measurement

Vacuum-packaged samples were placed in a 4 °C shelf life cooler (model F20T12WW, General Electric Co., Fairfield, Conn., U.S.A.) equipped with fluorescent lights (1200 LUX). LUX was measured with a calibrated Light Probemeter (model 403125, Extech Instruments, Burlington, Vt., U.S.A.). On days 1 and 8, vacuum packages were opened and chops were placed on nr 2 white foam trays (Koch Supplies, North Kansas City, Mo., U.S.A.) and over wrapped with 70 gauge oxygen permeable plastic (Pliant Corp., Uniontown, OH, U.S.A.). After chops had bloomed at room temperature (25 °C) for 30 min, color was measured on at least 4 locations using a Chroma Meter (model CR-200, Minolta Camera Co., Ltd., Osaka, Japan) calibrated to a white tile.

pH

The chop for protein analysis also provided sample for pH determination. Duplicate 1 g muscle samples were homogenized in 10-volumes of deionized water and pH was measured using a combination electrode on a digital pH meter (model 330, Orion Research Inc., Boston, Mass., U.S.A.).

Protein solubility

Total protein solubility was determined by homogenizing duplicate 1 g muscle samples with 10 mL of ice-cold 1 M KI/0.1 M potassium phosphate (pH 7.2) buffer in four 4-s bursts with a Kinematica polytron (Model PT 10/35, Brinkman Instruments, Inc., Westbury, N.Y., U.S.A.). The homogenate was incubated overnight on a rocker-mixer at 4 °C and then centrifuged at $2600 \times g$ for 30 min. The supernatant was decanted and protein concentration was measured using the biuret method (Gornall and others 1949). Sarcoplasmic protein solubility was similarly determined in 0.025 M potassium phosphate (pH 7.2) buffer. Myofibrillar protein solubility was calculated as the difference between total and sarcoplasmic protein solubility. Values are expressed as milligram protein per gram of muscle tissue.

Protein extraction and sample preparation

Whole-muscle protein extracts were prepared according to the modified procedures of Bechtel and Parrish (1983). A 0.5 g muscle sample was homogenized in 10 mL of extraction buffer (10 mM sodium phosphate buffer, pH 7.0, 2% [w/v] SDS) using a motordriven Dounce homogenizer. The homogenate was clarified by centrifugation (1500 × g) for 20 min at 20 °C. Protein concentration of the supernatant was determined using a modified Lowry assay (Lowry and others 1951) with premixed reagents (Bio-Rad, Hercules, Calif., U.S.A.). Samples were diluted to 6.4 mg/mL with water. One volume of the extract was then mixed with 0.5 volumes of sample buffer (3 mM EDTA, 3% [w/v] SDS, 20% [v/v] glycerol, 0.003% [w/v] pyronin-Y, and 30 mM Tris-HCl, pH 8) and 0.1 volume of β -mercaptoethanol. Samples were then denatured in a 50 °C water bath for 20 min, cooled on ice, and frozen at -80 °C until further analysis.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

Denatured protein samples were loaded (60 μ g protein/lane) onto a precast 15% polyacrylamide Tris-HCl (pH 8.6) separating gel with a 4% stacking gel. Broad range (6.5 to 200 kDa) molecular weight standards (Bio-Rad Laboratories) were run on each gel to determine protein band molecular weights and to account for gel-to-gel variation. Gels were run in duplicate on a Bio-Rad Criterion gel system for 15 min at a constant 100 V followed by 105 min at a constant 200 V at 4 °C. The running buffer contained 25 mM Tris (pH 8.3), 192 mM glycine, 2 mM EDTA, and 0.1% (w/v) SDS. The upper chamber also had 0.1% (v/v) β -mercaptoethanol added. Following electrophoresis, gels were stained with 0.05% (w/v) Coomassie brilliant blue R-250, 40% (v/v) methanol, and 7% (v/v) glacial acetic acid. Gels were destained in 15% (v/v) methanol and 7% (v/v) glacial acetic acid. Images of stained gels were captured using a KODAK Gel Logic 200 imaging system (Eastman KODAK Co., Rochester, N.Y., U.S.A.), and KODAK 1D Image Analysis software was used to measure band densities. Individual band densities were expressed relative to the density of the actin band.

Statistical analysis

All data were analyzed using the PROC MIXED procedure of SAS® (Version 9.2, SAS Inst. Inc., Cary, N.C., U.S.A. 2002–2008). The effects of HDP and aging on pH, WBSF, cook loss, EM, total moisture, color, solubility, and SDS-PAGE data were analyzed as a 3 imes2 split-plot factorial in a randomized block design. The model included treatment (control, HDP before processing, HDP after processing), aging (1 or 8 d), and the treatment by aging interaction as fixed effects and loin as a random block effect. The effect of HDP on processing parameters, TPA, and drip loss were analyzed with a model that included treatment as a fixed effect and loin as a random block effect. The LSMEANS and PDIFF options of SAS were used to identify significant differences (P < 0.05) between means.

Results and Discussion

Processing parameters

HDP treatment of muscle prior to brine injection and vacuum tumbling had an overall positive effect on the processing parameters of moisture-enhanced pork loins (Table 1). The percent injection amount verified that the targeted 10% pump rate was achieved for both HDP and control loin sections. Although the difference was not significant (P = 0.0963), HDP-treated loin sections tended to retain more brine through the tumbling process than controls. Furthermore, HDP-treated loin sections had lower (P < 0.05) brine loss with overnight equilibration and had a higher (P < 0.05) processing yield than controls.

HDP likely impacts retention of brine due to its effects on the integrity of the myofibrils. The ability of meat to take up and retain brine in moisture-enhanced products is a function of the WHC of the muscle, which is largely determined by the water-binding ability of the myofibrils (Offer and Trinick 1983; Xiong 2005). The expansion or contraction of the myofilament lattice impacts the amount of water that is retained in the muscle cells (Offer and Trinick 1983). The incorporation of phosphates into muscle has been shown to increase myofibril swelling and cause an expansion of the myofilamental lattice, thus allowing water-binding and physical entrapment of moisture (Xiong 2005). HDP causes myofibrillar fragmentation in the I-band region adjacent to the Z-lines (Zuckerman and Solomon 1998). The increased protein degradation and ultrastructural damage to the myofibrils associated with HDP likely impact myofilament spacing and myofibril swelling, which would in turn improve brine uptake and retention.

Texture characteristics

The effects of HDP and aging on the shear force measurements of moisture-enhanced pork loins are presented in Table 2. Aging from 1 to 8 d decreased (P < 0.0001) WBSF values approximately 19% in controls and 21% to 23% in HDP-treated loins. While HDP treatment numerically decreased WBSF values compared to controls on both days 1 and 8, the impact from the HDP treatments was not statistically significant. There was not a significant HDP treatment by aging interaction effect on WBSF in this study. Texture characteristics were also assessed using TPA measurements on day 2 (Table 3). Compared to controls, samples receiving HDP treatment after processing trended (P = 0.06) toward having higher springiness measurements. TPA measurements of hardness, cohesiveness, and chewiness were not influenced by HDP.

Based on WBSF data, previous research on nonenhanced pork and beef loins has demonstrated that HDP causes instantaneous

Table 1 - Means and standard error of means (SEM) for processing parameters of control and HDP-treated moisture-enhanced pork loins.

	Treatm	nent (<i>T</i>)			
	Control	HDP- treated ^A	SEM	Effects ^B	
Number of samples Injection amount (%)	24 9.98	24 10.49	0.24	NS	
Brine retained at the end of tumbling (%)	7.53	9.24	0.71	NS	
Brine lost during equilibration (%)	1.92ª	1.49 ^b	0.16	*	
Processing yield (%)	105.50 ^b	107.64ª	0.68	*	

ARepresent samples that were HDP treated before brine-injection and vacuum

tumbling. BMain effect significance: *significant at P < 0.05; NS = not significant (P > 0.05) 0.05). ab Means in the same row with different superscripts are significantly different

⁽P < 0.05).

Table 2— Means and standard error of means (SEM) for quality measurements of control and HDP-treated moisture-enhanced pork loins after aging (A) from 1 to 8 d.

	Treatment (T)									
	Control		HDP before processing		HDP after processing			Effects ^A		
	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8	SEM	T	A	$ extbf{ extit{T}} imes extbf{ extit{A}}$
Shear force (kg)	3.43ª	2.79°	3.35 ^{ab}	2.66 ^d	3.25 ^b	2.52 ^d	0.18	NS	****	NS
Meat pH	5.81ª	5.69°	5.80a	5.73 ^{bc}	5.77 ^{ab}	5.71°	0.03	NS	***	NS
Expressible moisture (%)	35.8a	31.2°	36.6ª	32.6 ^b	36.4ª	31.5 ^{bc}	0.6	NS	****	NS
Total moisture (%)	75.12°	75.02°	75.25 ^{bc}	75.64 ^a	75.56ab	75.18°	0.22	NS	NS	**
Cook loss (%)	24.5 ^{abc}	22.8 ^d	25.8ª	23.6 ^{cd}	25.6ab	24.2 ^{bcd}	0.5	*	****	NS
Lightness (L*)	49.36 ^b	49.25 ^b	50.25 ^a	48.87 ^b	48.77 ^b	49.82ab	0.54	NS	NS	**
Redness (a*)	6.57	6.97	7.08	6.96	7.09	7.19	0.23	NS	NS	NS
Yellowness (b*)	2.19 ^b	2.04 ^{bc}	2.49a	1.88°	2.14 ^{bc}	2.09 ^{bc}	0.19	NS	***	**
Chroma	7.01	7.28	7.61	7.22	7.45	7.51	0.25	NS	NS	NS
Hue	18.28ª	16.20 ^{bc}	18.57ª	13.60°	16.03 ^b	15.85 ^b	1.20	NS	****	***

AMain effect significance: *significant at P < 0.05; **significant at P < 0.05; **significant at P < 0.05; **significant at P < 0.001; ***significant at P < 0.001; ***significant at P < 0.001; NS = not significant (P > 0.05).

Table 3— Means and standard error of means (SEM) for texture profile analysis (TPA) of control and HDP-treated moisture-enhanced pork loins on day 2.

		Treatment (
	Control	HDP before processing	HDP after processing	SEM	Effects ^A
TPA-hardness	14.16	13.61	13.20	0.95	NS
TPA-cohesiveness	0.52	0.51	0.52	0.01	NS
TPA-springiness	0.55	0.56	0.58	0.01	NS
TPA-chewiness	3.85	3.64	3.78	0.21	NS

^AMain effect significance: *significant at P < 0.05; NS = not significant (P > 0.05).

Table 4 – Means and standard error of means (SEM) for cumulative drip loss measurements (% of initial sample weight) of control and HDP-treated moisture-enhanced pork loins.

	Control	HDP before processing	HDP after processing	SEM	Effects ^A
24 h	2.04	1.87	1.75	0.20	NS
96 h	4.32	4.02	3.67	0.27	NS
168 h	5.78	5.46	5.05	0.31	NS

 $^{\rm A}{\rm Main}$ effect significance: *significant at $P<0.05;~{\rm NS}={\rm not}$ significant (P > 0.05).

tenderization (20% to 50%) and enhances aging tenderization (Solomon and others 1997, 2002; Bowker and others 2008a, 2010). In the current study, the lack of dramatic reductions in WBSF due to HDP was likely the result of the tenderizing effect of the moisture-enhancement process. Numerous studies have shown that enhancing pork loins with a water/salt/phosphate solution decreases WBSF values compared to nonenhanced controls (Sutton and others 1997; Detienne and Wicker 1999; Brashear and others 2002: Prestat and others 2002a, 2002b: Brewer and others 2004; Sheard and Tali 2004; Baublits and others 2006; Hayes and others 2006). In the current study, nonenhanced controls were not utilized due to the limited size of the loin samples. However, the overall low shear values (<3.5 kgf) of the enhanced controls and the high shear values (mean of 5.9 kgf) of prescreening samples measured prior to freezing of whole loins (data not shown) suggests that the moisture-enhancement process decreased WBSF to values approaching background tenderness levels. Under these conditions, the tenderizing effect of HDP would not be as evident as previously observed in nonenhanced loins. The fact that HDP impact on texture characteristics (WBSF and TPA) was slightly greater when HDP was applied after enhancement is consistent with past data in which the degree of instantaneous tenderization with HDP was related to the postmortem time at which the treatment was applied (Paroczay and others 2002). The freezing and thawing of loins prior to HDP and aging treatments may have also contributed to the overall low WBSF values. This is supported by data demonstrating that the freezing/thawing of beef muscle increases tenderness (Winger and Fennema 1976; Solomon and others 2008). The tenderness improvements from days 1 to 8 in this study were consistent with past data showing the effects of aging on the tenderness of nonenhanced pork loins (Bowker and others 2010).

pH, WHC, and moisture characteristics

The effects of HDP and aging on the pH, WHC, and moisture characteristics of moisture-enhanced pork loins are presented in Table 2 and 4. Muscle pH was not influenced by HDP treatment but decreased (P < 0.01) with aging from days 1 to 8 in both controls and HDP-treated samples. The WHC of the samples was estimated by measuring the amount of moisture that could be expressed upon centrifugation (expressible moisture, EM) and by gravitational force (drip loss). In both control and HDP-treated samples, EM decreased (P < 0.0001) approximately 4% with aging from days 1 to 8 (Table 2). Expressible moisture was not significantly influenced by either HDP treatment. When analyzed as 3 treatments (control, HDP before processing, HDP after processing), cumulative drip loss was not significantly influenced by treatment after 24, 96, or 168 h of storage (Table 4). However, when the drip loss data from both HDP treatments were pooled, HDP-treated samples exhibited lower (P < 0.05) cumulative drip loss after 96 and 168 h than controls (data not shown). Of the total drip loss over the 168 h period, approximately 35% of the drip was lost during the first 24 h of storage, 40% was lost from 24 to 96 h, and 25% was lost from 96 to 168 h in both control and HDP samples. Total moisture measurements exhibited a significant HDP by aging treatment interaction (Table 2). With aging the moisture content of the controls did not change, whereas samples treated with HDP before processing had a small (approximately 0.4%), but statistically significant (P < 0.01) increase in moisture content with aging. Cook loss was shown to decrease (P < 0.0001) with aging from days 1 to 8. HDP samples, however, had a higher (P < 0.05) cook loss than controls.

While HDP treatment had a positive effect on brine retention in the moisture-enhanced loins, as evidenced by lower brine loss during equilibration and increased processing yields, HDP did not significantly impact the various measures of WHC in the final product. The drip loss data suggest that HDP may have a slight positive impact on WHC. This is in contrast to previous data on nonenhanced pork loins in which HDP was shown to cause a slight increase in drip loss after 1 d of storage (Bowker and others 2010). Comparing these 2 studies suggests that the phosphate in the brine may have a synergistic effect with the HDP treatment in regard to its impact on drip loss. The influence of the phosphate was also evidenced by the fact that moisture-enhanced loins in the current study had much lower cumulative drip loss measurements than loins from the previous study (Bowker and others 2010). Furthermore, drip loss occurred slower in moisture-enhanced loins. After 24 h of storage, nonenhanced loins had lost approximately 50% of their total drip (Bowker and others 2010), whereas enhanced loins only lost 35%. This is consistent with the well-established fact that phosphate incorporation into meat improves water binding and retention (Bendall 1954; Offer and Trinick 1983; Hamm 1986; Cannon and others 1993; Xiong 2005).

The decrease in the EM measurements from days 1 to 8 suggests that the WHC of the enhanced pork loins improved with aging. An improvement in WHC with aging has similarly been observed in nonenhanced pork loins (Van Laack and Smulders 1992; Joo and others 1995; Moeseke and Smet 1999; Bowker and others 2010). The "leaking out" hypothesis (Moeseke and Smet 1999) would suggest that the diminished amount of EM after aging is due to the fact that moisture lost by drip formation or evaporation at an earlier time postmortem cannot subsequently be lost at later times. Since the total moisture content of the samples in this study did not change substantially with aging; however, these data would support the idea of Kristensen and Purslow (2001) that improvements in WHC with aging are due to actual improvements in the waterbinding ability of the muscle and not the "leaking out" hypothesis. The improvements in the WHC with aging are likely due to ultrastructural and protein changes that occur as the result of the proteolysis associated with postmortem aging.

As previously discussed, the ability of muscle to retain moisture is largely dependent upon myofibrils and myofilament lattice spacing. There is shrinkage of interfilament spacing as muscle pH approaches the isoelectric point of myosin (pH 5) due to a reduction in the electrostatic repulsion between filaments (Hamm 1986). Since in the current study, muscle pH decreased with aging it is unlikely that muscle pH changes accounted for the apparent increase in WHC that was observed with aging in both the controls and HDP-treated samples. The ability of muscle to retain moisture is also reflected in the amount of fluid that is lost upon cooking. The decrease in cook loss with aging in the current study is consistent with the decrease in WHC that occurred with aging in the raw product. The amount of fluid lost during cooking is the result of structural changes brought on by the denaturation of proteins (Offer and Knight 1988). Even though HDP-treated samples had a higher cook loss than controls, the increased processing yield in HDP-treated

samples and the similarity in WHC measurements between HDP and control samples suggest that HDP treatment had a positive net impact on the overall yield of the moisture-enhanced loins.

Color characteristics

The effects of HDP and aging on the color of moisture-enhanced pork loins are shown in Table 2. Both L^* (lightness) and b^* (yellowness) measurements exhibited significant HDP by aging interaction effects. Values for L^* and b^* did not change with aging from days 1 to 8 in controls or in samples that were HDP treated after processing but decreased (P < 0.01) with aging in samples that were HDP treated before processing. Values for a* were not significantly affected by HDP or aging treatments. Although chroma values were not influenced by HDP or aging treatments, hue values exhibited a significant HDP by aging interaction effect. In both controls and samples treated with HDP before processing, hue values decreased (P < 0.0001) with aging from days 1 to 8. In samples treated with HDP after processing, however, hue values did not change with aging. Overall, it was observed that significant color differences (L^* , b^* , hue) between control and HDP-treated samples were only evident in day 1 samples but not day 8 samples.

Results from the current study suggest that HDP has minimal impact on color measurements in moisture-enhanced pork loins. This confirms previous observations made in nonenhanced pork loins treated with HDP (Bowker and others 2010). Studies on the color changes of moisture-enhanced pork loins with aging have shown similar results (Davis and others 2004). Although the current study exhibited some statistically significant changes in color measurements with treatments, the limited magnitude of these changes confirmed the visual observation that the treatments had no impact on the appearance of the pork loin chops.

Protein characteristics

Differences in pork quality attributes are closely associated with the extent of protein denaturation that has taken place during the postmortem period. Changes in protein solubility can be used to indicate differences in the degree of protein denaturation that has occurred in the muscle. Classes of pork loins with poor WHC and high drip loss exhibit lower sarcoplasmic, myofibrillar, and total protein solubility (Warner and others 1997). Myosin denaturation, which leads to diminished myofibrillar protein solubility, is thought to be the primary cause for the excessive drip loss observed in PSE (pale, soft, exudative) pork (Offer 1991). Thus, in the current study protein solubility measurements were used to determine the impact that HDP and aging may have on protein denaturation related to pork quality. The effects of HDP and aging on the protein solubility in moisture-enhanced pork loins are shown in Table 5. Myofibrillar protein solubility increased (P < 0.01) with aging from days 1 to 8 in both control and HDP samples. Compared to controls, neither HDP treatment impacted myofibrillar protein solubility. Neither HDP nor aging significantly influenced sarcoplasmic protein

Table 5 – Means and standard error of means (SEM) for protein solubility (mg protein/g tissue) of control and HDPtreated moisture-enhanced pork loins after aging (A) from 1 to 8 d.

			Treat	ment (T)						
	Control		HDP before processing		HDP after processing			Effects ^A		
	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8	SEM	T	Α	$T \times A$
Myofibrillar protein Sarcoplasmic protein Total protein	163.7° 48.7 212.4°	174.0ª 49.1 223.1ª	163.1° 51.5 214.6 ^{bc}	169.5 ^{ab} 50.2 219.7 ^{ab}	164.2 ^{bc} 49.7 213.9 ^c	171.7 ^{ab} 48.6 220.3 ^a	5.2 1.3 5.4	NS NS NS	NS *	NS NS NS

^AMain effect significance: *significant at P < 0.05; NS = not significant (P > 0.05). ^{abc}Means in the same row with different superscripts are significantly different (P < 0.05).

solubility measurements. Total protein solubility increased (P < 0.05) with aging in both control and HDP samples. HDP-induced changes in protein solubility are thought to be responsible for the shifts observed in protein content between the sarcoplasmic and myofibril fractions of muscle during isolation procedures (Spanier and Romanowski 2000). Data from previous studies using fresh beef and pork suggest that HDP increases myofibrillar protein solubility (Bowker and others 2008a, 2010) and support this hypothesis. The lack of HDP effect on protein solubility in the current study may be due to the overriding effect of the brine in the muscle. The increase in myofibrillar protein solubility with aging from days 1 to 8 is likely due to the proteolytic degradation of myofibrillar proteins that occurs with aging tenderization. Furthermore, the increased myofibrillar protein solubility observed after aging is consistent with the improved WHC of day 8 samples, as indicated by the decreased percentage of expressible moisture.

The effects of HDP and aging on the muscle protein profiles and protein degradation of moisture-enhanced pork loins were determined by SDS-PAGE. A representative gel of whole muscle protein extracts from one loin is depicted in Figure 1. The protein band densities (relative to actin band density) showing the strongest treatment effects are shown in Table 6. Based on comparisons with control samples, HDP had minimal impact on protein degradation as measured by SDS-PAGE analysis in this study. The protein profiles of both control and HDP-treated loins, however, exhibited strong aging effects between days 1 and 8. Bands corresponding to 135, 30 to 32, and 26 kDa increased (P < 0.01) with aging. Bands corresponding to 98, 60, 47, 41, and 38 kDa decreased (P < 0.01) with aging from days 1 to 8.

The aging related protein degradation that occurred in the moisture-enhanced pork loins was qualitatively very similar to that previously observed in fresh pork loins (Bowker and others 2010). The concurrent decrease in the 38 kDa band and the increase in the accumulation of a band in the 30 to 32 kDa range with aging most likely reflect the degradation of troponin-T (TnT). Numerous studies have documented that a 30 kDa TnT fragment accumulates with muscle aging that is closely associated with changes in tenderness (MacBride and Parrish 1977; Olson and others 1977; Penny and Dransfield 1979; Ho and others 1994; Huff-Lonergan and others 1996). Since protein extracts include both myofibrillar and sarcoplasmic proteins; however, it is also possible that the 38 kDa band also represents glyceraldehyde phosphate dehydrogenase, a sarcoplasmic protein known to degrade with postmortem aging (Okumura and others 2003; Bowker and others 2008b). Compared to previous data on fresh pork loins (Bowker and others 2010), how-

ever, the relatively low abundance of the 30 to 32 kDa TnT fragment in aged samples in this study seems to indicate that less proteolysis occurred with aging. From the current study, it cannot be definitively determined if the decreases in the 98, 60, 47, or 41 kDa bands with aging are due to proteolysis or due to changes in protein extractability. Similarly, the increase in the density of the 135 kDa protein band with aging is either due to the accumulation of degradation products from larger myofibrillar proteins or due to increased protein extractability. The fact that myofibrillar protein solubility increased with aging and that proteolysis was not extensive, as evidenced by the low accumulation of the 30 to 32 kDa TnT

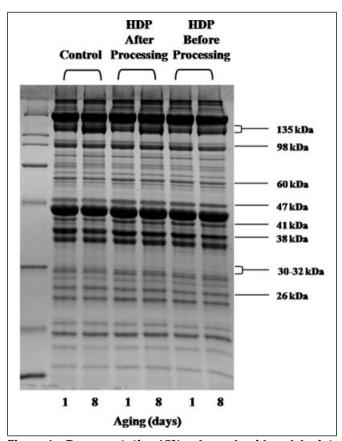


Figure 1 - Representative 15% polyacrylamide gel depicting the effects of hydrodynamic pressure processing (before and after brine-injection and vacuum tumbling) and aging on whole-muscle protein extracts from moistureenhanced pork loins.

Table 6 – Means and standard error of means (SEM) for SDS-PAGE protein band intensity measurements of control and HDP-treated moisture-enhanced pork loins after aging (A) from 1 to 8 d.

			Treat	ment (T)						
	Control		HDP before processing		HDP after processing			Effects ^B		
	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8	SEM	T	Α	T * A
135 kDa	0.593°	0.851ª	0.556°	0.734 ^b	0.559°	0.740 ^{ab}	0.055	NS	****	NS
98 kDa	0.177a	0.152 ^b	0.164ab	0.154 ^b	0.167 ^{ab}	0.150 ^b	0.012	NS	*	NS
60 kDa	0.138bc	0.129°	0.154a	0.139abc	0.149ab	0.132°	0.014	NS	*	NS
47 kDa	0.220ab	0.192 ^b	0.219ab	0.191 ^b	0.222a	0.199 ^b	0.010	NS	***	NS
41 kDa	0.335ª	0.298 ^b	0.362ª	0.303 ^b	0.337ª	0.307 ^b	0.020	NS	****	NS
38 kDa	0.453ab	0.384°	0.469ª	0.400 ^{bc}	0.470a	0.391°	0.031	NS	****	NS
30-32 kDa	0.070 ^{bc}	0.092a	0.074 ^b	0.093 ^a	0.064°	0.092a	0.012	NS	**	NS
26 kDa	0.250 ^b	0.283ª	0.241 ^b	0.261 ^{ab}	0.245⁵	0.279ª	0.023	NS	***	NS

ASDS-PAGE protein band intensity expressed as net band intensity relative to the intensity of the actin band.

BMain effect significance: *significant at P < 0.05; **significant at P < 0.01; ***significant at P < 0.001; ***significant at P

fragment, suggests that changes in protein solubility and extractability may have played a significant role in the SDS–PAGE observations.

The degree of proteolysis that did occur with aging from days 1 to 8, however, was sufficient to impact meat quality. The proteolytic degradation of key myofibrillar and cytoskeletal proteins with aging was likely responsible for the improved tenderness and WHC that was observed in day 8 samples. Previous studies have suggested that the proteolytic degradation of myofibrillar proteins may enhance WHC and reduce drip loss (Morrison and others 1998; Kristensen and Purslow 2001; Lonergan and others 2001; Huff-Lonergan and Lonergan 2005; Bee and others 2007). In the current study, the strong effect that aging had on protein degradation, tenderness, and WHC compared to HDP supports the link between proteolysis, WHC, and tenderness.

Conclusions

This study investigated the impact that HDP and aging have on the processing parameters and the final product quality of moisture-enhanced pork loins. When applied prior to brine-injection and vacuum tumbling, HDP improved brine retention and processing yield. Whether applied before or after processing, HDP demonstrated potential to improve the texture and WHC of moisture-enhanced pork loins with minimal impact on the visual appearance of the final product. Overall, data from this study demonstrate for the 1st time that HDP may have beneficial effects on the processing and final product quality in brine-injected, moisture-enhanced pork products.

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